Separation of Cellulose From Rice Husk Treated With Ionic Liquid Prior To Enzymatic Treatment

by

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Dissertation submitted in partial fulfillment of

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(Chemical Engineering)

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CERTIFICATION OF APPROVAL

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A project dissertation submitted to the

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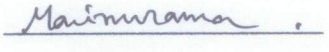
Approved by,



(Dr. Muhammad Moniruzzaman)

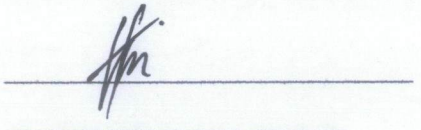
UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

September 2013

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or person



ZULHAFEEZ BIN ZAPAR

ABSTRACT

The present work deals with the use of multiple-step procedures to obtain cellulose from rice husk. The objective of this study is to provide new environmentally friendly and efficient method to separate higher percentage of cellulose from the rice husk with minimal structural alteration. The method comprised of applying ionic liquid for pretreatment prior to enzymatic delignification of rice husk biomass. Lignocellulosic biomass pretreated with ionic liquid is known to be favorable for subsequent enzymatic hydrolysis due to their reduced cellulose crystallinity and decreased lignin content. Delignification was done to remove the lignin from the lignocellulosic material so that the rice husk obtained from the treatment will be of higher cellulose composition. Therefore, in this study it was hoped to achieve as maximum cellulose yield as possible without damaging the environment. The effect of ionic liquid to the enzymatic treatment was examined, together with the structure of rice husk biomass, lignin content and enzymatic yield.

ACKNOWLEDGEMENTS

First and foremost, I would like give praises to the Almighty for the opportunity to complete this project. Even though I have poured my effort towards the completion of this project, it would not have been possible without the support given by many individuals. Hence, I would like to extent my acknowledgement to all of them who had been involved directly or indirectly in this project.

I am highly indebted to my final year project supervisor, Dr. Muhammad Moniruzzaman for his exemplary guidance and supervision, especially during the times when I have doubts and inquiries regarding the project. The constant help and support given by her has helped me a lot during the accomplishment of the project.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Agricultural production creates a significant amount of agricultural waste. Some of it is recycled, while large amounts remain unused causing disposal problem. Uncontrolled burning in the fields is not only a hazardous disposal solution but it is also wasting useful energy. Rather than disposing the waste it is better to utilize the waste as raw biomass material as it has high potential for manufacturing biodegradable products. Biomass is a carbon based material composed of a mixture of organic molecules containing carbon and hydrogen. It usually possesses atoms of oxygen and nitrogen whilst including small quantities of other elements, such as metals. The carbon used to construct biomass is absorbed from the atmosphere as carbon dioxide (CO2) by plant life, using energy from the sun, making it as the most abundant renewable resource available (Holm and Lassi, 2011). There are advantages in the use of biomass material. A major potential source of alternative fuels is biofuels produced from cellulosic biomass, which have a number of potential benefits. The raw materials used in the production of cellulosic biofuels are largely waste materials from agriculture, forestry, or other non-food crops. The use of wastes overcomes the problems of using food and feed grains, such as corn, for biofuel production. Cellulosic biofuels also help reduce greenhouse gas emissions relative to fossil fuels.

Rice husk (RH) is one of the major agricultural residues generated as a by-product during rice milling process. The Food and Agricultural Organization of the United Nations (FAO) forecasts that the global rice production stands at around 486 million tonnes on milled basis in 2012 (FAO,2012). Rice production has managed to

establish a dominant agriculture industry worldwide, where it is the most widely consumed staple food for a large part of the world's human population. With such a large availability of raw materials, it becomes economical to make them as sources of more applications. In the present, RH usually ends up being burned in open spaces as it have small commercial value, thus causing environmental pollution and disposal problems (Rafiee et al. 2012). Due to the need to conserve energy and resources, efforts have been made to burn the husk under controlled conditions and to utilize the resultant ash as building, semiconductor, composite, and abrasive materials. For example, in the village of Patelli in the north-eastern Bihar state, electric distribution system is powered by the waste product of rice husks. The rice husk is poured into a hopper about 100 pounds per hour, and gassified to run a simple turbine to generate electricity. The resultant ash of rice husk burning is an active catalyst and a good material for catalyst support.

Rice husk is a source of lignocellulose which the major constituent consist of lignin, cellulose and hemicellulose. These components can be recovered for further applications by combining suitable physical, chemical, physico-chemical and biological treatment. However, the rick husk cell contains layers that differ in structure and chemical composition. Direct conversion of untreated rice husk through enzymatic treatment usually results in low product yield because of its recalcitrant nature attribute. Hence, pretreatment that disrupts the lignocellulose biomass is necessary to enhance the reactivity of the lignocellulosic material with the enzyme. Physical, chemical and a combination of physical/chemical pretreatments are the commonly employed methods in pre-treating lignocellulose biomass.

In order to address this issue, ionic liquid will be used for the pretreatment. Ionic liquids with cellulose-dissolving ability offer a novel solution for pre-treating lignocellulose biomass. Various ionic liquids, such as 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) [11], 1-ethyl-3 methylimidazolium acetate ([EMIM][OAc]) [12], and 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM] [DEP]) (Dadi et al., 2006) have been applied as solvents in pretreatment step before enzymatic saccharification to enhance reducing sugars yield (Ang et al. 2012). The treated rice husk will later be characterized physically and chemically, and compared with untreated rice husk.

1.2 Problem Statement

Until now, the most commercially fully utilized natural resource containing cellulose is wood, and it is expected that supply of wood at a reasonable price will be insufficient in the future. Apart from that, the agricultural byproduct such as rice husk will become more attractive as sources of cellulose. Millions of unused wastes are produced from agriculture causing disposal problem and pollution when they are burnt. It will be useful to consider using rice husk, providing positive effect to the environment.

However, it takes a long time to extract the cellulose from the rice husk and other components such as lignin are also still available after treatment. Most of the methods require high temperature and pressure as well as concentrated chemical. This will result in degradation of products, pose environmental hazard and not cost effective. Therefore it is desired to come up with a pretreatment process which is not only environmentally friendly but also efficient and cost effective for the biomass conversion to cellulose.

1.3 Objective

The objective of the research is to provide a new environmentally friendly and efficient method with ionic liquid to separate cellulose fibres from rice husk biomass with minimal structural and chemical alteration. The aim is to overcome the low delignification efficiency associated with the difficulties to in enzyme accessibility to the solid substrate and the poor substrate and product solubility in aqueous system.

1.4 Scope of Study

To achieve the stated objectives, the project is divided into 2 phases, and in the second phase, the work is as stated in Table 1.

Table 1: Project Execution Phase

Project Phase 2	- Carry out lab work - Chemical and physical characterization of treated and untreated rice husk - Documentation

The project focuses on the delignification efficiency after ionic liquid pretreatment. The composition of lignin in the rice husk will be decreased after treatments. The factors which will maximize the efficiency of the method will be analysed so that the project will come up with the most effective and clean approach to separate the cellulose fibres from rice husk. The effect temperature during pretreatment and amount of ionic liquid during pretreatment is studied. Also the effect of the pretreatment to the treated sample is studied by observing the difference of end result (after enzymatic delignification) between treated and untreated rice husk.

CHAPTER 2

LITERATURE REVIEW

2.1 Cellulose

Majority of biomass on the planet is made up of cellulose and it is the primary component of all cell walls (Leland et al. 2006). It is the most abundant natural polymer on planet, given its presence in plants and its widespread use for ropes, sails, paper, timber for houses and other application (Simone et al. 2012). For this reason, cellulose has always been playing an important role in human's life. Methods for fabrication of cellulose substrate go way back to the early Chinese dynasties, even during Egyption pharaoh's time (Pe´rez and Mazeau, 2005). For example, fine cotton and lingerie are found the pharaoh's tomb. In the current modern world, it still is one of the important raw materials used in many industries. In fact, cellulose and its derivatives are one of the principal materials of use for industrial exploitation (paper, nitrocellulose, cellulose acetate, methyl cellulose, nano cellulose, etc.). Cellulose has proved its market and represents a promising economic investment. Cellulose, with the formula [$(C_6H_{10}O_5)$n where n = repeating units] is the world's most common natural polymer, a long chain made by the linking of glucose monosaccharide units. It is an organic compound, a polysaccharide consisting of linear chain of several hundreds to thousands of D-glucose, a simple sugar (Kalia et al. 2009). Generally it can be found in plant material (in plant cell wall), but it can also be found in some bacteria with the ability to produce cellulose such as Acetobacter.

Cellulose chemistry began with the discovery of sugar of cell walls published by French Scientist, Anselme Payen in 1838. His research solved the mystery regarding the variable composition of cell walls in different plants. After purifying

the cell wall of various plant samples, Payen found that the remaining material have the same elemental composition as a starch.

A natural polymer in nature, cellulose is a long chain created by linking of smaller molecules. The links in cellulose chain consist of sugar β-D-glucose. The sugar units are linked when water is eliminated by combining the H and –OH group. Linking of two of this sugar produced a disaccharide called cellobiose. In the cellulose chain, the glucose unit are in 6 membered rings called pyranoses. They are joined by single oxygen atoms between C-1 of pyranose ring and the C-4 of the next ring. Cellobiose is consisted of 8 free alcohol groups, one acetal linkage and one hemiacetal linkage. Since a molecule of water is lost because of the reaction of an alcohol and a heliacetal to form an acetal, the glucose unit in the cellulose polymer is called anhydroglucose unit. The hydroxyl at C-4 can approach carbonyl at C-1 from either side, resulting in different stereochemistry at C-1. When the hydroxyl group at C-1 is on opposite side of ring as C-6 carbon, it is said to be in β configuration, as in cellulose. With all functional groups in equatorial position, the β configuration causes the molecular chain of cellulose to extend in a straight line, making it a good fiber-forming polymer (Kalia et al. 2009). The structure of cellulose is shown in figure 1 as shown below.

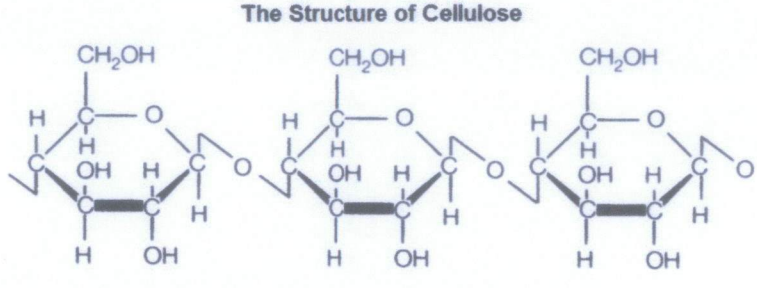


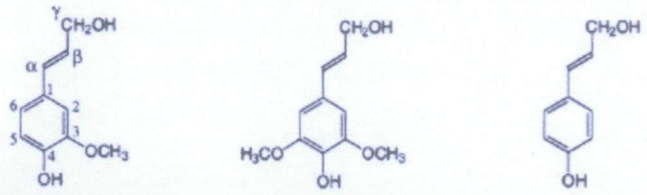
Figure 1: Structure of Cellulose

Because of the equatorial positions of the hydroxyl, they extend laterally along the molecule and are readily available for hydrogen bonding. The hydrogen bonds caused the chains to group together in a highly structured manner. The

interchain hydrogen bonds in the crystalline area are very strong given the good strength of the resultant fibre and insolubility in most solvents. The hydrogen bonds also caused the cellulose to have thermoplastic properties. However, in the less structured region the chains are far apart and more available for hydrogen bonding with other molecules like water. It can absorb large quantities of water, thus making the cellulose swell. Although swelling because of the water, it does not dissolve in water.

In living plants cellulose occurs in fibers, consisting of repeated crystalline structures resulted from the aggregation of cellulose chains, termed microfibrils (Panaitescu et al. 2011). It constitutes the basic framework of the cell conveying a great resistance to tensile forces (Pe´rez and Mazeau, 2005). The microfibrils are found in the cell wall, enclosed by amorphous non-cellulosic matrix, which are hemicellulose and lignin. Lignin keeps the water in fibers, acts as a protection against biological attack and as a stiffener to give stem its resistance against gravity forces and wind. Hemicellulose found in the natural fibers is believed to be act as a compatibilizer between cellulose and lignin (Kalia et al. 2009). Each fibre has a complex, layered structure which consists of a thin primary wall; the first layer deposited during cell growth encircling secondary wall. The first wall deposited after cell division is called middle lamella and is composed of pectic material. The secondary wall is made up of three layers and the thick middle layer is the one that determine the mechanical properties of the fibre.

The cellulose microfibrils represent about 20-30% of dry weight cell wall material occupying about 15% of cell wall volume (Pe´rez and Mazeau, 2005). The orientation and disposition of microfibrils in the wall are important because this more or less control the capacity of the wall to deform and the direction in which the deformation can occur (Pe´rez and Mazeau, 2005). In the final stage of cell wall differentiation especially in the middle lamella and primary cell wall, other wall poymer (lignin) are incorporated into the spaces around polysaccharide fiblar elements to form lignin polysaccharide. Lignin emerges from enzymic dehydrogenative polymerization of three phenylpropanoid units, which are coniferyl alcohol, sinaphyl alcohol and coumaryl alcohol (Stenius 2000).



trans-Coniferyl alcohol *trans*-Sinapyl alcohol *trans-p*-Coumaryl alcohol

Figure 2: Lignin Structural Unit

It constitute between 10-30% of the dry weight of wood, which make them second to cellulose. Lignin contributes to the mechanical strength of the plant cell wall and confers resistance to pathogen. They also confer resistance to water due to their hydrophobicity and control solute transport and water content.

On the other hand, hemicellulose constitutes a large number of different polysaccharide molecules which actually form a matrix for the cellulose microfibrils. This involves molecular interaction such as hydrogen bonds and van der Waals forces. In addition to structural properties, hemicellulose other functions are in cell signalling or as precursor to cell signalling. There are two major hemicellulose polysaccharide available which are xyloglucans and arabinoxylans. Xyloglucans is the predominant hemicellulose in dicotyledons while arabinoxylan is predominant in graminaceous monocotyledons.

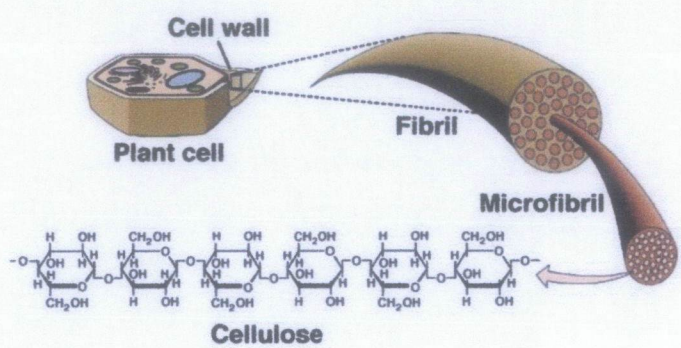


Figure 3: Microfibril in plant cell wall

Because of the extensive intra and inter-molecular hydrogen bonding, cellulose does not readily dissolve in typical aqueous solvents, relatively stable polymer, and crystalline in nature (Shukla et al. 2013; Eichhorn et al. 2010). Its insolubility has made it extremely difficult to be extract from plant cell wall. Strong chemical treatment including acid and alkali is required to remove other cell wall components to yield cellulose preparation. Thus, as is being analysed, the cellulose sample may have already been modified by the harsh chemical extraction treatment.

The potential use of cellulose is widely extended especially nanocellulose. Polymer composites with micro or nano cellulose fibers have a bright future as material for glass-fiber-containing composites in many industrial fields like automotive, construction, electronics, biomedicine, cosmetics, and last but not least, the packaging industry. Among the advantages of using cellulose fibers as reinforcements in polymer composites renewability, low cost, low density, low energy consumption, high specific strength and modulus, high sound attenuation, low abrasivity and relatively reactive surface are the most important (Panaitescu et al. 2011).

2.2 Rice Husk

Rice husk (RH) is the hard protecting coverings of grains of rice. It is a byproduct of rice hulling industry, amongst the few agricultural residues that can be readily obtained in huge amount in one location. During the milling process, the husks are removed from the grain to create brown rice; the brown rice is then milled further to remove the bran layer to become white rice (Syuhadah and Rohasliney, 2011). The structure of rice grain is shown below in figure 4.

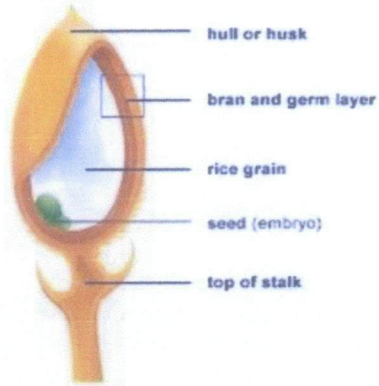


Figure 4: Structure of rice grain

RH represents approximately 20% by weight of rough rice, where approximately one-fifth of the annual gross rice production in the world accounts for RH (Kumar, 2012; Rafiee et al. 2012). This means that approximately 97 million tonnes of rice husk is produced globally on milled basis. Most of the rice husk is used for animals bedding material and burned for energy generation, while its industrial application is still limited (Leiva et al. 2007). Only a small amount of rice husk is utilized as fuel for bimass gasification, steam boilers and electricity generation. Rice husk ash has also emerges as low cost and pure silica and silicates used in chromatograph separation technology, detergent, cements and steel industries (Kalapathy et al., 2002). Another application of rice husk is as biosorbent for metal ions removal (Krishnani et al., 2008). The organic part of rice husk is usually used for production of acetic acid, xylose,furfural, xylitol and ethanol (Chandrashekar et al., 2003). Figure 5 below shows the burning of the waste from rice milling which is rice husk.

Figure 5: Burning of rice husk

Like other agricultural waste, RH is quite fibrous and requires relatively little input energy to be processed into various products such as panel board (Ndazi et al. 2007). It is also widely available at relatively low cost. RH has the same basic components as wood but in different percentage. It contains 25–35% cellulose, 8–21% hemicelluloses, 26–31% lignin, 15–17% amorphous silica and waxes, and 2–5% of other soluble substances (Leiva et al. 2007).

RH is one of the lignocellulosic residues that have attracted much attention among researchers due to its relatively high cellulose content and its potential to be used in biofuel production. However, direct conversion of untreated rice husk usually results in low product yield as plant cell wall is naturally recalcitrant to the biological degradation (Ang et al. 2012). The high crystallinity of cellulose makes it recalcitrant to hydrolysis into its individual glucose subunits (Dadi et al. 2006). Biological treatment with enzymes can be performed under mild reaction conditions, but this method is very slow in aqueous system due to the difficulties of the enzyme accessibility to the solid substrate and poor solubility of lignin. Therefore, pretreatment is necessary to overcome the recalcitrant structure to achieve efficient delignification of the lignocellulosic biomass.

The worldwide annual rice husk output is about 97 million tonnes and over 97% of the husk is genertated in developing countries. As Malaysia is one of the producers, according to Malaysian Ministry of Agriculture 408000 metric tonnes of rice husk are produced each year. As the rice husk is burnt, it releases carbon dioxide

to the atmosphere. Carbon dioxide is one of the greenhouse gases which caused greenhouse effect. The increase in temperature of the world is contributed by global warming which are caused by the greenhouse effect. Greenhouse effect is a process in which the emission of infrared radiation by the atmosphere warms a planet surface.

It is important to find a use to the rice husk as it is one of the major contributor to air pollution. For instance, in 1992, the New Sunday Times reported that indiscriminate burning of paddy husk in the rice fields of Kedah caused air pollution and also posed health hazard to the community as well. For many years the children there have been afflicted with skin and eye irritation caused by the polluted air. In Cairo, the rice husk burning problem is so severe that seasonal black cloud appears specifically in fall due to the rice husk and straw burning. It is so thick that it is possible to be spotted from an airplane. During the harvesting month, the smog can become unbearable even to for the residents who have been accustomed to the polluted air. The black cloud is dangerous for plane landing operation as the pilot view is blocked and breathing the polluted air is extremely unhealthy for humans and other living things alike.

2.3 Pretreatment Methods for Biomass Material

Over the years, a large number of pretreatment approaches have been investigated on wide variety of biomass sample. However, many pretreatments, such as dilute acid hydrolysis , pH controlled liquid hot water treatment, and lime result in biomass substrates that lack components (often hemicellulose), but cellulose remain crystalline after pretreatment. Enhancement of hydrolysis with pretreated cellulose was seen in early studies by Ladish et al. (1978) where he used Cadoxen (mixture of 5% cadmium oxide in 28% aqueous ethylene diamine. Cellulosic materials were softened in cadoxen for 12 hours in room temperature, rinsed and saccharified using cellulose. Saccharification reaction rates were enhanced compared to untreated ones. The authors contributed their findings to the disruption of cellulose crystalline structure. However, long incubation time, toxicity of cadoxen solvent and difficulty in recovery and reuse of the diamine limit this type of pretreatment route. Similar results is also found by incubating the cellulose first in a solution of an

iron/sodium tartrate complex followed by addition of a concentrated sodium hydroxide solution (iron precipitates as hydroxide) (Hamilton et al., 1984).

In recent study, phosphoric acid at concentrations ranging between 70 and 81% was shown to swell and dissolve cellulose at higher concentrations (Wood, 1988). In another study by Zhang et al. (2006), the author used ice-cold concentrated phosphoric acid to dissolve cellulose followed by its precipitation by diluting the mixture with ice-cold water. In this experiment, the enzymatic hydrolysis rate was found to be increased. Cellulose swelling and dissolution with other concentrated acids (sulphuric acid, hydrochloric acid, and nitric acid) have also been reported (Hudson and Cuculo, 1980). Acid pretreatment normally require corrosion resistant material of construction and some mineral acids produce degradation product (Mosier et al., 2001).

2.4 Pretreatment with Ionic Liquid

The goal of pretreatment is to break the lignin shield and the crystalline structure of cellulose whilst increasing the porosity of the cellulose. It is considered as a very important tool for practical cellulose conversion processes. The pretreatment of cellulosic materials can affect its physical properties such as its degree of polymerisation, its crystallinity and even the surface area of the substrate accessible in the case of further enzymatic hydrolysis (Olivier-Bourbigou et al., 2010). Several pretreatment strategies to extract lignin from lignocellulose with high yield have been designed and successfully developed in the past. These include physical (limited pyrolysis and mechanical disruption/comminution), physiochemical (steam explosion, ammonia fiber explosion), chemical (acid hydrolysis, alkaline hydrolysis, high temperature organic solvent pretreatment, oxidative delignification) methods. These extraction methods have one main disadvantage: the lignin starts to degrade after a certain amount of lignin is extracted. In addition, they are tailored to a specific type of lignocelluloses (Mosier et al., 2005). Cellulose solvents should have most of the features listed below (Olivier-Bourbigou et al., 2010). They have to:

1. be able to dissolve cellulose at low temperatures,
2. be non-volatile, non-toxic and chemically stable,
3. no decompose cellulose,

4. be easy to regenerate,

5. be recyclable,

6. be cost effective and easily processed

7. non-toxic to enzymatic and microbial fermentation.

In the list above, it indicates that solvent use and recovery increases the total cost of pretreatment. Some pretreatment methods are too slow (even weeks), and these methods cannot be considered as "green" since hazardous or toxic compounds are released. Therefore, more efficient pretreatment procedures are required. One potential alternative is the use of ionic liquids.

Lignocellulosic biomass pretreated with ionic liquid (IL) is favorable for subsequent enzymatic hydrolysis due to their reduced cellulose crystallinity and decreased lignin content (Dadi et al. 2006). Their ability to dissolve large amounts of cellulose at considerably mild conditions and the feasibility of recovering 100% of the used IL to its initial purity makes them attractive (Heinze et al. 2005). ILs are a group of organic salts that are liquids near room temperature. They are completely ionic with most ionic liquids consisting of an organic cation and an inorganic anion. Many ionic liquids are also liquids at room temperature making it an ideal solvent to work with. ILs can dissolve biomass materials with heating, while at room temperature, ionic liquids such as 1-ethyl-3-methylimidazolium acetate (EmimAC) act as a swelling agent of woody biomass instead of dissolution (Lucas et al., 2010). With their low volatility, fluidity at ambient temperatures, and unique solvent properties, ILs comprise a class of prospective solvents that are potentially ''green'' due to their minimal air emissions (Dadi et al. 2006). ILs are characterized by properties such as negligible vapour pressure and non-flammability under ambient conditions, high thermal conductivity, a wide electrochemical window and high polarity. They also have the ability to dissolve a wide diversity of materials, including salts, fats, proteins, amino acids, surfactants, sugars, polysaccharides and organic solvents (Siedlecka et al. 2011).

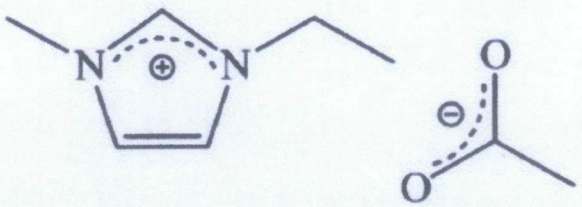


Figure 6: 1-ethyl-3-methylimidazolium acetate structure

The main objective of rice husk pretreatment is to alter the structure of the cellulosic biomass. This caused the solid substrate to be more accessible to the adsorbates (enzyme and/or chemical) during enzymatic delignification. The lignin seal of the cellulose must be interrupted and only then can the crystalline structure of cellulose be disrupted.

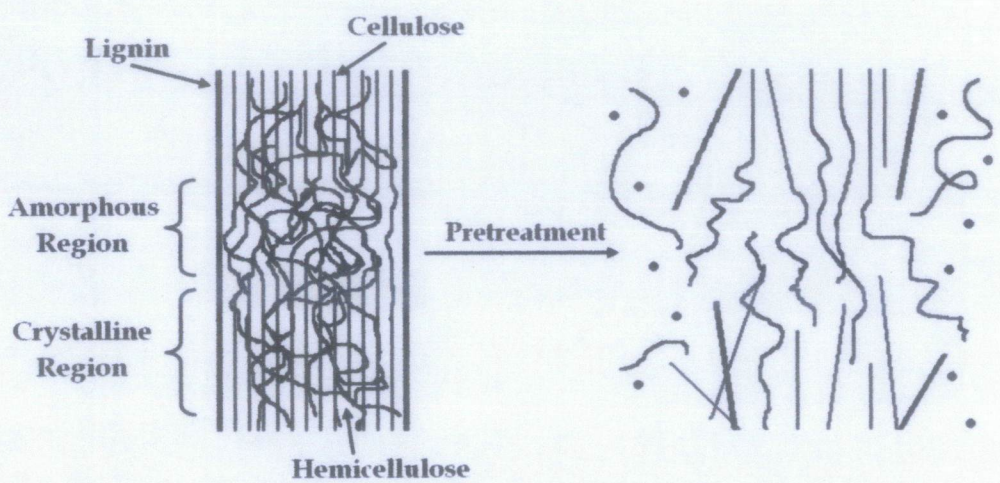


Figure 7: Pretreatment effect on cellulose crystalline structure adopted from Mosier et al., 2005.

Cheng et al., 2012 found that the cellulose lattice expands and distort prior to full dissolution in [C2mim][OAc]. It is observed that strong hydrogen bond interactions that occur between [C2mim][OAc] and cellulose disrupt the intermolecular hydrogen

bonds between cellulose molecules. This is the advantage from one of ILs attributes; Hydrogen's bonding ability of the IL anion (Olivier-Bourbigou et al., 2010). The OAc forms H-bond with the hydroxyl group of the biomolecule. In other studies made upon pretreatment using IL, from the attenuated total reflectance Fourier transform-infrared (ATR FT-IR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy (SEM) results, the regenerated cellulose were more amorphous, less crystalline, and possessed higher structural disruption compared with untreated rice husk (Ang et al. 2011). Initial rates of enzymatic hydrolysis of regenerated cellulose were at least 50 times that of untreated cellulose when pretreated with 1-n-butyl-3-methylimidazolium chloride (Dadi et al. 2006). This suggests the potential of ILs for rice husk pretreatment before undergoing enzymatic treatment to obtain maximum amount of cellulose.

2.5 Enzymatic Delignification

Bleaching releases high levels of lignin in wastewater which treatment demands rising costs for elimination of more and more difficult pollutants to isolate (Zhang et al., 2002). Therefore elimination of lignin through cost-effective and nature friendly methods to replace traditional expensive and polluting procedures have being showing increasing interest (Sena-Martins et al., 2008). Usage of enzyme in pulp and paper industry is investigated and the multiple potential applications of laccase in this field of industry define this particular enzyme as a promising alternative to existing costly and polluting processes (Barreca et al., 2003; Shi, 2006).

Lignin is strongly linked with other cell wall components. It minimizes the accessibility of cellulose and hemicellulose to microbial enzymes. Delignification will help in the removal of lignin from lignocellulosic material. The removal of lignin open the way for colonization by other microbial population. This process is usually done by natural enzymatic or industrial chemical processes. The enzyme laccase will catalyse the oxidation of lignin by transferring one electron leading to the formation of the aryloxy radical, which via a second one electron transfer yields a variety of products. This oxidation process is coupled to the reduction of dioxygen to water by a four-electron transfer. Non-phenolic substrates are more resistant to

oxidized directly by laccases, either because they are too large to penetrate into the enzyme active site. This limitation can be overcome using mediator. The presence of mediator increases laccase effective substrate range to include non-phenolics by reducing effective redox potential. The small mediator molecule is first oxidized or activated by the laccase, and then the mediator penetrates the compact lignocellulosic structure more easily than the enzyme and thus realizes the oxidation of lignin inside the sample (Xueyu et al., 2013).

CHAPTER 3

METHODOLOGY

3.1 Experiment Method

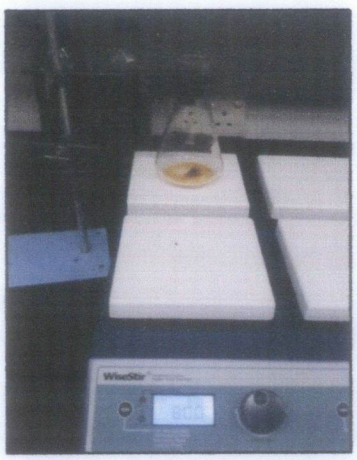


Figure 8: Ionic Liquid Pretreatment; Heating of rice husk mix with ionic liquid

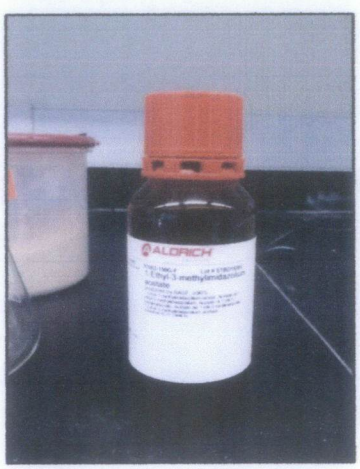


Figure 9: Ionic Liquid: 1-Ethyl-3-methylimidazolium acetate

Experimental Procedure

1. The rice husk is crushed using grinding machine until the particle size become 500 μM particle size.
2. The rice husk is then dried in the oven at 105°C overnight.

3. 200mg of rice husk is added to 500mg ionic liquid in a one neck flask.
4. Heat it at 80°C with magnetic stirring for 1 hour. Check the temperature every 10 minutes.

5. The treated rice husk, lignin and ionic is recovered. The treated rice husk is washed with acetone-water mixture (ratio 1:1) and several times with distilled water. Then, the pretreated rice husk is dried.

6. After cooling the rice husk-Ionic liquid mixture, sodium acetate buffer containing laccase is added to the flask and also 1-hydroxybenzotriazole which act as a mediator. Carry the reaction with magnetic stirrer at 50°C .

7. After cooling the reaction mixture to room temperature, 0.1M NaOH is used to wash away the ionic liquid and lignin from the treated rice husk.
8. Wash it one more time with distilled water until pH becomes neutral.

9. The treated rice husk is dried for 24 hours at 65°C in oven.

10. The treated rice husk is weighed.
11. Analyze/Characterize the treated rice husk.

Repeat the experiment with different:

1. Size of rice husk (range 500 - 700 nm)
2. Temperature during pretreatment
3. Amount of ionic liquid (range 0.5 – 1.0 g)

3.1.1 Ionic Liquid Pretreatment and Delignification

In a typical experiment, 0.2 g of the rice husk (500nm) and 0.5g IL were placed in a flask and heated at 80°C for 1 hour. After cooling the rice husk-IL mixture to room temperature, water-acetone (1:1) were added as anti-solvent and stirred at room temperature for 20mins to separate the solid materials from dissolved lignin and IL. After allowing the mixture to settle, the treated rice husks were recovered.

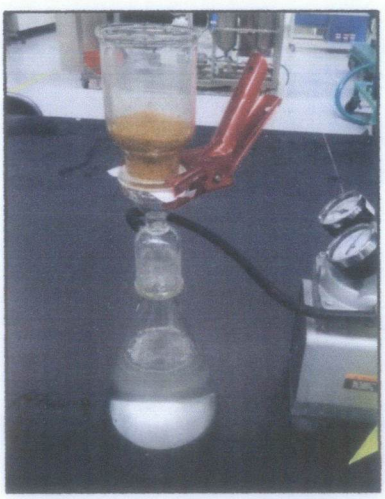


Figure 10: Vacuum Filtration

The treated rice husks were washed several times with distilled water to remove residual IL. The treated samples were then weighted to check the weight. Dried treated rice husk and 10ml phthalate buffer (pH 4.5) were placed in a flask. Laccase (5 wt% biomass) was added to the flask. Reactions were carried out at 50°C with magnetic stirring, with or without ionic liquid. After 24 hours, 0.1M NaOH was added and the mixture was stirred for 1 hour to extract lignin from the enzymatically delignified IL treated rice husk. The mixture was filtered under mild vacuum and CRF's were collected. To remove traces of NaOH, the CRFs were washed with distilled water until the wash water became neutral. The CRFs were oven dried at 65 °C for 24 hours to constant weight.

Figure 11: Sample after enzymatic delignification

3.1.2 Characterization Of Untreated And Treated Materials

a) Powder X-Ray diffraction

- The crystallinity of the untreated and treated rice husk materials was investigated by X-Ray Diffraction (XRD)

b) Fourier Transform Infrared Spectroscopy

- The FTIR spectra of samples were recorded from a KBr disk containing 1% of finely ground sample on an FTIR Spectrophotometer. The spectral outputs were recorded in transmittance mode as a function of wave number

c) UV determination of solubilized lignin

- One mililiter of supernatant (collected after enzymatic delignification) was transferred into a 5ml vial and diluted with 4 ml of NaOH 0.1M. The absorbance was measured at 280nm

3.2 Key Milestone

3.2.1 Final Year Project 2 (FYP 2)

Table 2: Key milestone (FYP 2)

Event or Deliverable	Target Date
Submission of Progress Report	Week 8
Pre – SEDEX	Week 11
Submission of Draft Report	Week 12
Submission of Dissertation (Soft Bound)	Week 13
Submission of Technical Paper	Week 13
Oral Presentation	Week 14
Submission of Dissertation (Hard Bound)	Week 15

3.3 TOOLS

3.3.1 Lab equipments

- One neck flask, beaker, magnetic stirrer, pipette, clamp with stand, filter funnel, heater, oven, vacuum filter

3.4 CHEMICALS AND MATERIAL

3.4.1 Rice husk

- The lignocellulosic material used to obtain cellulose. Rice husk is obtained from Universiti Teknologi Petronas (UTP) laboratory in its raw form.

3.4.2 1-Ethyl-3-Methylimidazolium Acetate

- Ionic liquid (IL) use for rice husk pretreatment. It is highly effective as the IL has higher basicity compare to others. Purchased from Sigma Aldrich Inc.

3.4.3 Laccase (from Trametes versicolour)

- Enzyme use for enzymatic delignification. Purchased from Sigma Aldrich Inc.

3.4.4 Phthalate (pH 4.5)

- Buffer Solution.

3.4.5 Distilled Water

- For washing/cleansing purpose.

3.4.6 0.1M Sodium Hydroxide

- To wash the lignin away from the rice husk after enzymatic delignification.

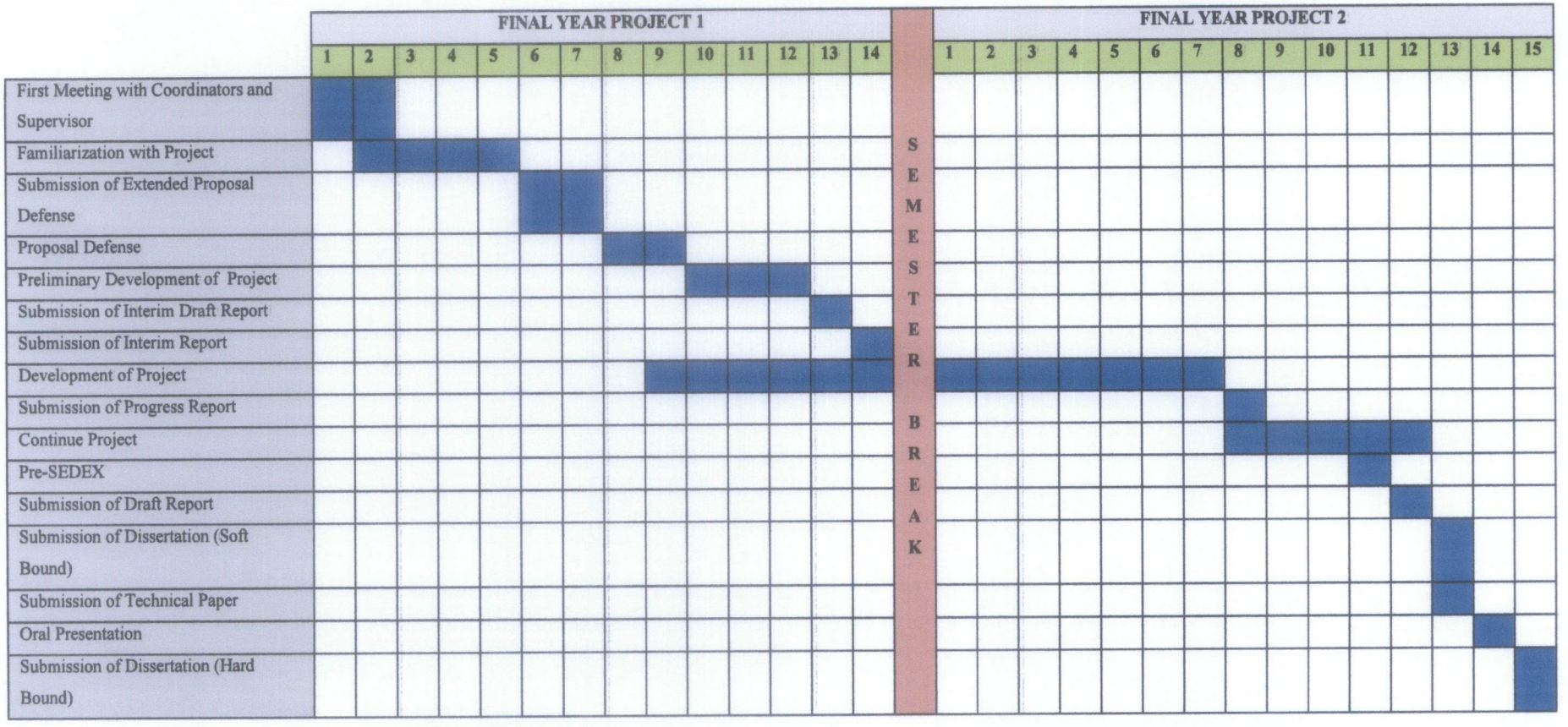
3.4.7 Acetone

- For washing purposes

3.5 Gantt Chart

The gantt chart is as below.

Table 3: Gantt Chart



CHAPTER 4

RESULT & DISCUSSION

4.1 Result

Table 4: Result for rice husk ionic liquid pretreatment

Sample	Size (nm)	Temperature (°C)	Amount of IL (g)	Weight before (g)	Weight after (g)	Heating duration (hour)	Moisture Content after Pretreatment (%)
1	500	45	0.5	0.2	0.16	1	12.5
2	500	80	0.5	0.2	0.17	1	6.41025641
3	500	80	1	0.2	0.17	1	5.93349068
4	500	60	0.5	0.2	0.18	1	6.22589208
5	500	70	0.5	0.2	0.17	1	5.86358906
6	500	95	0.5	0.2	0.17	1	4.587155963
7	700	60	0.5	0.2	0.17	1	1.470588235
8	700	70	0.5	0.2	0.18	1	1.834862385
9	700	50	0.5	0.2	0.18	1	5.113636364
10	700	80	0.5	0.2	0.17	1	5.349356847

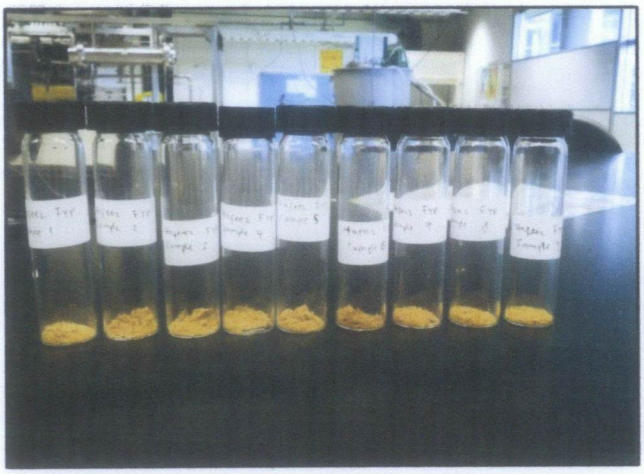


Figure 12: Sample after pretreatment

4.2 Discussion

Commercial laccase was selected as biocatalyst because it can degrade lignin of biomass without disrupting other components (Blanchett, 1991). It was also reported that laccase can maintain their activity for the oxidation of 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) and cathechol in ionic liquid –aqueous system containing over 80% of water (Shipovskov et al., 2008).

In particular, BASF, one of the pioneers in industrial usage of ILs has relied on 1-ethyl-3-methylidazolium acetate [C2mim][OAc] for dissolution of cellulose because of its desirable properties such as low toxicity ($LD_{50} > 2000 mg kg^{-1}$), low corrosiveness, low melting point, low viscosity (10mPa s at 80°C), and favourable biodegradability. Because of this properties, [C2mim][OAc] was selected as the ionic liquid to be used in the ionic liquid pretreatment.

After weighing the samples it is found that the weight of the rice husk samples decreased after ionic liquid pretreatment as shown in Table 3. Small fractions of the pretreated rice husk are loss during the recovery phase which is washing the rice husk after pretreatment. It was assumed that the weight loss is not due to solubilization of of cellulose, but only due to lixiviation of the rice husk as it would require harsher processing. After the pretreatment like ionic liquid pretreatment on wood, the swelling of the cell wall was occurred due to ability of

ionic liquid to disrupt hydrogen bond network between cellulose and lignin. After the pretreatment, it is assumed a small fraction of lignin is loss in the process as the structure of cell wall opens up just enough to cause a small amount of lignin to go out. In addition to weight loss after pretreatment, water content in the rice husk samples play a key role in determining its solubility in ionic liquids. Overall, water is found to significantly reduce the solubility of rice husk in ionic liquid (Kilpelainen et al., 2007). It was very difficult to remove the water effectively from the rice husk samples. This provides ample justification on why it is only possible to partially solubilized the rice husk in ionic liquid with presence of water inside the sample. The moisture content of the samples varies a little bit from each other. This occurred because of the duration of the sample put into the incubator were not controlled. When weighing the treated sample after pretreatment and enzymatic delignification it was better to weigh the sample after its temperature is back to room temperature to achieve same condition as weighing before treatment. Kilpelainen et al. (2007) found that kinetics of the process of the treatment process were dependent on temperature of treatment. At higher temperature, it was found that all of the examined wood samples dissolved relatively rapidly. With varying the temperature, the optimum temperature will be determined. Further analysis was not completed due to equipment failure and long queue to use the equipment. The analysis of sample characteristics should be done in future to determine the efficiency of ionic liquid better. The analysis should comprise as table 4 below:

Table 5: Result for Enzymatic Delignification

Delignification Method	With Ionic Liquid Pretreatment	Without Ionic Liquid Pretreatment
Crystallinity		
FTIR Spectra		
Weight Loss		
Lignin Loss		

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion and Recommendation

A new environmental friendly and effective method to separate cellulose fibres from rice husk biomass using ionic liquid pretreatment prior to enzymatic delignification was studied in this project. The aim was to overcome the low delignification efficiency associated with the difficulties to in enzyme accessibility to the solid substrate and the poor substrate and product solubility in aqueous system. It achieve as maximum cellulose yield as possible without damaging the environment and with minimal structural and chemical alteration to the cellulose structure. The effect of ionic liquid to the enzymatic treatment was examined, together with the structure of rice husk biomass, lignin content and enzymatic yield. The approach can be used for improving method to separate cellulose from lignocellulosic material in the future. The project opens up a novel approach for separation of cellulose from rice husk. The separation method will be significantly improved if the lignin degradation with ionic liquid pretreatment is used at the right condition with minimal structural and chemical alteration.

For future studies, there are some recommendations suggested. The rice husk is needed only in a small amount to perform the experiment, but we can obtain it from UTP in a big amount, so there is no need to outsource the material. Next, while doing the experiment, check the temperature every 10 minutes while heating to ensure uniform temperature. Also use vacuum filtration to obtain the sample after washing fast as the typical filtering process using filer paper without vacuum condition is very slow. Last but not least, consult the lab technician earlier to confirm booking for sample analysis as the equipment is not available in big quantity but the

users are many. It is better to book early as most of the time the delay of a project is caused by waiting for turn to use the equipment.

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